

Gene silencing: RNA makes RNA makes no protein

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A mutation that disrupts post-transcriptional gene silencing in *Neurospora crassa* has been found to affect the homologue of a plant-encoded RNA-dependent RNA polymerase. This enzyme may produce a specificity determinant of gene silencing and mediate an epigenetic conversion at the RNA level.

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‘Quelling’ in *Neurospora crassa* is similar to genetic interference in nematodes, cosuppression in plants and, quite possibly, antisense RNA effects in many organisms [1]. In each of these phenomena, the introduction of a foreign nucleic acid activates a sequence-specific mechanism of RNA turnover. The target RNAs of this turnover mechanism are similar to the foreign nucleic acid. In quelling, for example, the presence of a transgene based on the *albino 1* (*al-1*) gene results in sequence-specific degradation of the endogenous *al-1* RNA [1]. In nematodes, the introduction of double-stranded RNA produces a phenotype of mutations in genes similar to the introduced RNA [2]. There are other similar examples in plants and, collectively, these phenomena are referred to as post-transcriptional gene silencing, although ‘RNA silencing’ may be a more accurate term. Now, through the genetic analysis of quelling in *Neurospora*, new light has been shed on the mechanism of post-transcriptional gene silencing [3].

Specificity of post-transcriptional gene silencing

To explain the nucleotide sequence specificity of quelling and the other examples of post-transcriptional gene silencing it has been proposed that there is an antisense RNA corresponding to the foreign nucleic acid [4]. The models to explain the origin of this hypothetical antisense RNA invoke either DNA or RNA templates (Figure 1) [5]. In the DNA template models, the foreign nucleic acid is a transgene that is directly transcribed to form the antisense RNA. In some of these examples, with antisense transgenes, the antisense RNA is transcribed by design. In other examples, a sense orientation construct has rearranged as it integrated into the genome so that the transcripts are of the antisense RNA. In a third variation on the DNA template model, the transgene has integrated in the plant genome close to an endogenous promoter, but in reverse orientation so that antisense RNA is produced by transcription from the endogenous promoter. The

alternative models invoking RNA templates require that the antisense RNA is transcribed from the sense RNA of the foreign nucleic acid. By definition, an RNA synthesis enzyme using an RNA template would be an RNA-dependent RNA polymerase.

Until recently there has been no firm evidence to favour any of these models. But with the recent characterisation of the *quelling defective* (*qde-1*) mutation in *Neurospora*, there is now a good reason to favour the RNA template models. It transpires that *qde-1* encodes a homologue of a tomato RNA-dependent RNA polymerase [3,6]. This discovery must be satisfying for Lindbo, Dougherty and coworkers who, in an influential early paper on post-transcriptional gene silencing [7], first proposed the involvement of the host encoded RNA-dependent RNA polymerase.

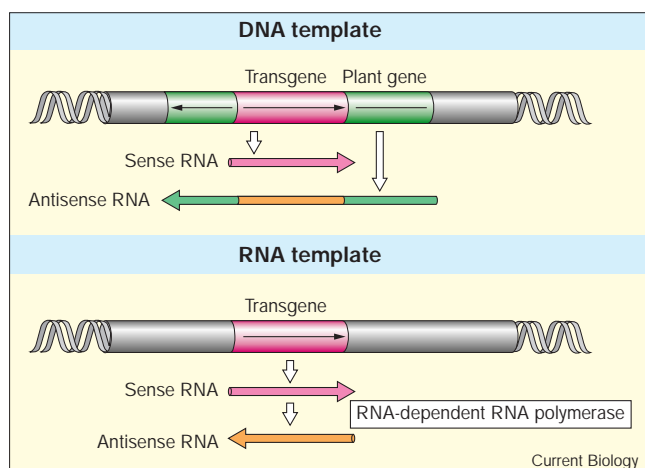
The mechanism of post-transcriptional gene silencing

Although the product of *qde-1* has been identified, there is still some way to go before post-transcriptional gene silencing is fully understood. For example, it is not clear how the *qde-1* product or its tomato homologue could function as an RNA polymerase, because these proteins lack the conventional RNA-dependent RNA polymerase motifs [3,6]. Perhaps these proteins represent a completely novel type of RNA-dependent RNA polymerase. Alternatively, it remains possible that the RNA-dependent RNA polymerase is a heteromeric complex in which the Qde-1 protein is a non-catalytic subunit.

A second unanswered question concerns the template of the RNA-dependent RNA polymerase. Is this a double-stranded RNA, as indicated by work on genetic interference in nematodes [2]? The ability of combined sense and antisense transgenes in plants to induce post-transcriptional gene silencing [8] is also consistent with the involvement of double-stranded RNA. Involvement of double-stranded RNA would also explain the potent ability of RNA viruses, which have a double-stranded replicative intermediate, to activate post-transcriptional gene silencing in plants [9]. It would not, however, explain why base-paired regions of endogenous RNA do not activate post-transcriptional gene silencing.

A further uncertainty concerns the nature of the RNA produced by the RNA-dependent RNA polymerase. It is unlikely that the RNA-dependent RNA polymerase produces an RNA that is as abundant, or as long, as a typical mRNA, because it would have been detected in analyses of RNA in post-transcriptional gene silencing. Instead, it is possible that the RNA-dependent RNA polymerase

Figure 1



Models for the production of antisense RNA in plants exhibiting post-transcriptional gene silencing. Antisense RNA could be produced by transcription from the transgene DNA; in the example shown a plant promoter located adjacent to the transgene locus directs transcription of the antisense RNA. Alternatively, the antisense RNA could be produced from the sense transgene RNA by an RNA-dependent RNA polymerase. (See text for details.)

product is short and present at a low level. The precise role of this hypothetical RNA is also unclear. It is possible, for example, that it is an antisense RNA that anneals to the homologous sense RNA and thereby provides a target for a double-stranded RNase [4]. It is also possible that the interaction with antisense RNA affects RNA abundance indirectly by interfering with translation or, for viral RNA, by blocking replication.

Epigenetics and RNA-dependent RNA polymerase

Many examples of post-transcriptional gene silencing are epigenetic. For example, in nematodes injected with double-stranded RNA, the resulting genetic interference spreads beyond the injected cell but is not inherited [2]. There is a similar situation in transgenic plants in which the foreign nucleic acid responsible for inducing post-transcriptional gene silencing is a virus vector [10]. In these plants, provided that the virus and the transgene are similar at the nucleotide level, there is post-transcriptional gene silencing targeted against the transgene and viral RNAs. Initially, the virus vector accumulates but, eventually, both the viral and transgene RNAs are eliminated from the cells. The silenced state persists in these cells, despite the absence of the inducing virus, but it is not maintained by a genetic change as it is not inherited in progeny of the infected plant [10].

One explanation of these epigenetic phenomena invokes an interaction at the DNA or chromatin level and, consistent with this idea, it is known that there are RNA–DNA

interactions associated with post-transcriptional gene silencing. These interactions lead to sequence-specific methylation of the corresponding DNA sequences [10]. At first it seems anomalous that a post-transcriptional process is associated with interactions at the DNA level. If the RNA–DNA interaction can interfere with transcription, however, it is not difficult to envisage the connection between events at the DNA and RNA levels. The transcriptional interference might lead to the production of aberrant transcripts of the transgene. This RNA could then mediate maintenance of post-transcriptional gene silencing in the absence of the foreign nucleic acid that initiated the process. In effect these aberrant RNAs would be a secondary form of the foreign nucleic acid that is required for post-transcriptional gene silencing.

There is, however, one anomalous observation that is inconsistent with this model of epigenetic gene silencing. This concerns a system of virus-induced gene silencing in peas carrying a transgene based on pea seedborne mosaic virus, which has an RNA genome [10]. When the transgenic plants were inoculated with pea seedborne mosaic virus there was post-transcriptional gene silencing manifested as reduction of the transgene mRNA and elimination of the viral RNA from the infected plants. There was also resistance against secondary infection with pea seedborne mosaic virus. The anomalous feature of this system lies in the specificity of the resistance, which is based on the sequence of the viral inducer of silencing rather than the transgene [11]. Thus, in plants with a transgene based on the DPD1 strain of the virus, infection with DPD1 induced resistance against DPD1 and not against the NY strain, but infection with the NY strain induced resistance against both DPD1 and NY.

To account for this result it is necessary to invoke a specificity determinant of post-transcriptional gene silencing which is derived from the virus inducer of gene silencing and which persists in the absence of the viral RNA. The involvement of host-encoded RNA-dependent RNA polymerase in post-transcriptional gene silencing provides an explanation for how this RNA-based epigenetic process could operate: initially, the RNA-dependent RNA polymerase would copy part of the viral RNA; subsequently the copy RNA would be a template for the RNA-dependent RNA polymerase and, if the process is repeated through several cycles, this host-encoded enzyme would be replicating parts of the viral RNA.

RNA-dependent RNA polymerase regulation

It has been known for a long time that, in plants, virus and viroid infection leads to an increase in RNA-dependent RNA polymerase activity and mRNA levels [6]. Interestingly, the level of *qde-1* mRNA is higher in an *al-1* quelling strain of *Neurospora* than in an untransformed strain [3], suggesting that the regulation of RNA-dependent RNA

polymerase mRNA may be a general feature of post-transcriptional gene silencing irrespective of whether the foreign nucleic acid is a transgene or a virus. If that is the case, the post-transcriptional gene silencing mechanism must be even more complex than had been anticipated. Presumably, the surveillance system that detects foreign nucleic acid will not only have the ability to determine the specificity of RNA degradation, but will also include a mechanism for regulating the production of RNA-dependent RNA polymerase.

The natural role of post-transcriptional gene silencing

Homologues of *qde-1* are not only present in plants and *Neurospora*. There are recognisably similar genes in the fission yeast *Saccharomyces pombe* — but apparently not in the budding yeast *Saccharomyces cerevisiae* — and a nematode [3] and, consequently, it seems likely that post-transcriptional gene silencing operates in a range of organisms. Interestingly, in neither of the papers describing the RNA-dependent RNA polymerase sequence [3,6] is there a reference to mammalian homologues. This apparent absence of mammalian homologues may be related to the natural role of post-transcriptional gene silencing that, in plants at least, is as an antiviral defense system. Perhaps post-transcriptional gene silencing became redundant in animals when antiviral mechanisms evolved that were based on the immune system and interferon-related pathways. There is, however, one report of post-transcriptional gene silencing in mammalian cells [12], and it cannot be ruled out that a mammalian *qde-1* homologue exists but is not yet represented in the public sequence databases or is difficult to detect because of sequence divergence.

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